Validation and application of the 3D human reconstructed skin micronucleus assay (RSMN) using the EpiDerm™ tissue to the safety assessment of cosmetics ingredients

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Abstract

The Reconstructed Skin Micronucleus assay (RSMN) using EpiDerm™, is a more realistic model for evaluating the genotoxic potential of dermally applied chemicals. The assay exhibits good transferability and inter- and intra-laboratory reproducibility. In Phase 3, the assay protocol was modified (e.g., extension of the treatment from 48h to 72h) and an additional 12 coded chemicals were subsequently tested. There was an excellent overall specificity (87%) with only few mispredictions. Six true positive chemicals were initially negative using a 48h dosing regimen but were positive when tested in a 72h dosing regimen. The inclusion of a 72h dosing regimen increased the sensitivity to 80%. Two of the 3 chemicals missed by the 72h regimen (2-AAF and CaCl₂) were Ames positive and were picked up by the 3D skin Comet assay. These data support the use of the RSMN assay for follow-up of positive results from standard in vitro assays and therefore removing the need for in vivo follow-up testing.

Methods

Dose tissue topically

1. Precipitation at the beginning and the end of the treatment period is noted.
2. Keratinocytes are released by trypsinization.
3. Medium contains Cytochalasin B to allow monitoring of nuclear division.
4. Analysis of micronuclei.
5. Prepare slides for MN scoring.

Dose tissue topically

Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase 1-3 testing</th>
<th>Bridging studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>62.5%</td>
<td>88.0%</td>
</tr>
<tr>
<td>Specificity</td>
<td>95.2%</td>
<td>87.0%</td>
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<tr>
<td>Overall concordance</td>
<td>86.2%</td>
<td>89.5%</td>
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</tbody>
</table>

Conclusions

- An international validation study with 38 coded chemicals shows a high sensitivity (80%) and specificity (87%) for the prediction of in vivo genotoxicity outcomes.
- Two of the compounds missed are Ames positive and would be picked up by the 3D skin Comet assay, which increases the sensitivity to 92%.
- The data supports the use of the human 3D skin-based genotoxicity assays for follow-up of unfavourable results from standard in vitro assays (e.g., Ames, micronucleus) and therefore is a direct replacement of in vivo follow-up testing.


This work was sponsored by Cosmetics Europe.